

PHYLOGENETIC RELATIONSHIPS OF THE CHRYSOBALANACEAE INFERRED FROM CHLOROPLAST, NUCLEAR, AND MORPHOLOGICAL DATA¹

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ABSTRACT

The Chrysobalanaceae, a pantropical family containing about 525 species, has often been nested within the Rosaceae despite evidence for recognizing it as a separate family. In 1963, Prance clearly placed Chrysobalanaceae as a distinct family containing 17 genera. However, the family has been linked with various other families and orders and recently has been placed within the order Malpighiales. Because of these discrepancies, a phylogenetic analysis for the family was launched to examine its monophyly and to investigate the relationships within the Chrysobalanaceae as well as its relationships to other groups. Comparative phylogenetic analyses were performed using morphological, *rbcl*, and ITS sequences. The data sets were analyzed independently and in combination. After exploration for hard incongruencies among the independent data sets, a simultaneous analysis of all the data was completed. The combined analysis resulted in a resolved, supported topology with several unambiguous morphological synapomorphies. The resulting topology indicated that the family is a well-defined monophyletic group that is sister to *Euphronia* Mart. & Zucc. (Euphroniaceae). The present tribal groupings, however, are paraphyletic.

Key words: Chrysobalanaceae, Chrysobalanaceae, Couepieae, Hirtelleae, ITS, morphology, Parinariaceae, *rbcl*.

The Chrysobalanaceae R. Br. includes 17 genera and about 525 species. Most of these species occur in the lowlands of the tropics and subtropics, and the family is especially well represented in the New World tropics. The present family circumscription places the 17 genera in four tribes: Chrysobalanaceae, which includes *Chrysobalanus* L., *Grangeria* Comm. ex Juss., *Licania* Aubl., and *Parastemon* A. DC.; Couepieae, which includes *Acioa* Aubl., *Couepia* Aubl., and *Maranthes* Blume; Parinariaceae, which includes *Bafodeya* Prance ex F. White, *Exelodendron* Prance, *Hunga* Prance, *Neocarya* (DC.) Prance ex F. White, and *Parinari* Aubl.; and Hirtelleae, which includes *Atuna* Raf., *Dactyladenia* Welw., *Hirtella* L., *Kostermanthus* Prance, and *Magnistipula* Engl. (Prance & White, 1988) (Table 1). All species of Chrysobalanaceae are woody, and most are trees or shrubs. The vegetative architecture of the plants is relatively uniform. The flower, by contrast, is comparatively diverse, although nearly every genus is characterized by an underlying uniformity of inflorescence and floral structure. The flowers are bisexual, rarely unisexual, and markedly perigynous. The flower size and shape vary within wide limits, from minute

patelliform flowers (*Licania elaeosperma* (Mildbr.) Prance & F. White) scarcely larger than a pinhead, to tubular flowers that are longer than 10 cm (*Maranthes gabunensis* (Engl.) Prance). Floral symmetry varies from almost completely actinomorphic to strongly zygomorphic. In most genera, the entrance to the receptacle tube contains numerous long straight retrorse hairs. There are always five, completely free sepals that range from slightly to strongly imbricate. Petals, when present, are always five and inserted on the margin of the disc, and are mostly caducous; however, they are absent in more than half of the species of *Licania*. The number of stamens also varies from two in *Parastemon urophyllus* (Wall. ex A. DC.) A. DC. to more than 300 in some species of *Couepia*.

The ovary is fundamentally composed of three carpels, which are united only by the gynobasic style, but in most species only one carpel is functional. The fruit is basically a dry or fleshy drupe of varying size. The interiors of the fruit are often densely hairy, and the endocarp is variable, often containing a special mechanism for seedling escape (Prance, 1963; Prance & White, 1988).

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Table 1. Genera and number of species of tribes of Chrysobalanaceae (from Prance & White, 1988).

Tribe/genera	No. of species
Chrysobalanaceae	
<i>Chrysobalanus</i>	2
<i>Grangeria</i>	2
<i>Licania</i>	192
<i>Parastemon</i>	2
Parinarieae	
<i>Bafodeya</i>	1
<i>Exellodendron</i>	5
<i>Hunga</i>	11
<i>Neocarya</i>	1
<i>Parinari</i>	44
Couepieae	
<i>Acioa</i>	4
<i>Couepia</i>	67
<i>Maranthes</i>	12
Hirtelleae	
<i>Atuna</i>	11
<i>Dactyladenia</i>	27
<i>Hirtella</i>	103
<i>Kostermantus</i>	2
<i>Magnistipula</i>	11

The family is locally important in the tropics for fruit, construction materials, fuel, charcoal, folk medicine, and shade trees. Several species are cultivated. *Chrysobalanus icaco* L. (coco plum), for example, is tinned and bottled as syrup in Colombia and Venezuela. *Couepia rufa* Ducke and *C. bracteosa* Benth. fruits are found in markets in Brazil. *Parinari macrophylla* Sabine (gingerbread plum) and *P. curatellifolia* Planch. ex Benth. (mobola plum) are both eaten in Africa. *Parinari curatellifolia* is used in beer making, and a red dye is extracted from its young leaves in West Africa. In Brazil, *Licania tomentosa* (Benth.) Fritsch is widely planted as an avenue tree providing shade and *C. icaco* is widely used as an ornamental in southern Florida.

The taxonomic position of the Chrysobalanaceae has been particularly controversial. The majority of authors of general systems of classifications who treat Chrysobalanaceae as a family distinct from Rosaceae leave it in Rosales. Among other opinions, close affinities have been proposed for the Chrysobalanaceae with a diversity of taxonomic groups: Dichapetalaceae and Trigoniaceae (Hallier, 1923), Geraniaceae and Tropaeolaceae (Hauman, 1951), and Connaraceae (Gutzwiler, 1961). Many of these families' and orders' circumscriptions have changed in the last few years, and more recently the family has been placed in the rosid clade and sometimes in the order Malpighiales based solely on molecular data (Soltis et al., 2005).

Cronquist (1981, 1988) placed Chrysobalanaceae in a much more widely conceived order of Rosales, including 24 families. He acknowledged that the Rosales formed an exceedingly diverse order. However, he thought it was more useful to delimit the order broadly than to fragment it and lose sight of the interrelationships among its parts.

In 1989, Dahlgren placed the family into Theales under the superorder Theanae, whereas Thorne, in 1999, placed the family in its own order, Chrysobalanales, under the superorder Rosanae.

According to Prance and White (1988), the embryological differences are great among the Chrysobalanaceae, Rosales, and Theales. During 25 years of studying Chrysobalanaceae, Prance and White found no evidence pointing unequivocally to an evolutionary relationship between the Chrysobalanaceae and any other family.

The preliminary attempts to apply mainly morphological data to a cladistic analysis at the family level by Prance and White (1988) were thwarted because of the widespread occurrence of parallelism and because only a few of the many characters used at this time satisfied the requirements for inclusion in a cladistic analysis. Chappill (1992) carried out a cladistic analysis, using the 1988 monograph by Prance and White, to determine the level of parallelism in the family and to see whether or not the Prance and White system was supported. The results of the analysis support a monophyletic family; however, many of the tribal groupings of Prance and White were not well supported.

Phylogenetic work using molecular data within this family is in its infancy. A large molecular phylogenetic study using *rbcL* sequence data (Chase et al., 1993) places the Chrysobalanaceae in the rosid clade, with Trigoniaceae as the sister group. A study using 18S ribosomal RNA (rRNA) sequence data (Soltis et al., 1997) also placed the family in the rosid clade. Both of these studies only used one species of *Chrysobalanus* and *Licania*. The combined analysis of *rbcL*, *atpB*, and 18S data sets (Soltis et al., 2000) and the analysis of the same three genes plus *nd1B-C* (Davis et al., 2005) housed the family in the Malpighiales within the eurosid I clade or, more recently, what has been called the Fabidae (Cantino et al., 2007) or the "fabids" (Judd & Olmstead, 2004). Other recent studies have examined one to three Chrysobalanaceae taxa with two to three genes and have also found the family positioned in the Malpighiales (Davis & Chase, 2004; Tokuoka & Tobe, 2006; Soltis et al., 2007); however, more taxa from different sources of DNA are needed to confirm this placement. Because of these discrepancies between and within the traditional taxonomy and the phylogenetic analyses, further studies using phylogenetically

more informative characters and more taxa from this family are warranted.

To test potentially competing hypotheses of family circumscription and generic relationships in the Chrysobalanaceae, two DNA regions that evolve at different rates were sequenced and a morphological data set was constructed. The first DNA region was the chloroplast gene *rbcL*, and the second was the nuclear region ITS.

The present study carries out a phylogenetic analysis with the following purposes: (1) to test the monophyly of Chrysobalanaceae and their relationships with the Malpighiales (APG II, 2003; Davis & Chase, 2004; Davis et al., 2005; Tokuoka & Tobe, 2006; Soltis et al., 2007) using an expanded *rbcL* analysis, and (2) to investigate the internal generic relationships within the Chrysobalanaceae using *rbcL*, ITS sequencing, and morphological and anatomical studies.

MATERIALS AND METHODS

INGROUP SAMPLING

The most recent systematic treatment of Prance and White (1988) has been followed as the basis for generic circumscriptions of the family. The genus *Parastemon* was not included in the analysis because of lack of material.

OUTGROUP SELECTION

Outgroups varied depending on the analysis performed and material available. For the larger *rbcL* analysis, the following species were used: a single species of *Balanops* Baill. (Balanopaceae), *Cornus* L. (Cornaceae), *Connarus* L. (Connaraceae), *Euphronia* Mart. & Zucc. (Euphroniaceae), *Ochna* L. (Ochnaceae), *Comesperma* Labill. (Polygalaceae), *Spiraea* L. (Rosaceae), *Stylobasium* Desf. (Stylobasiaceae), *Cadellia* F. Muell., *Guilfoylia* F. Muell., *Suriana* L. (Surianaceae), *Tetracoccus* Engelm. ex Parry, and *Androstachys* Prain (Picrodendraceae); two species of *Trigonia* Aubl. (Trigonaceae) and *Tapura* Aubl.; and three species of *Dichapetalum* Thouars (Dichapetalaceae). A subset of the most closely related taxa were used for a smaller *rbcL* analysis. For the ITS analysis, however, only *Dichapetalum* and *Euphronia* were used because of amplification problems. *Dichapetalum* and *Tapura* were chosen for the morphological analysis. These taxa were selected on the basis of the *rbcL* analysis of Chase et al. (1993) and Litt and Chase (1999), and various historical observations mentioned above.

DNA EXTRACTIONS

The total genomic DNA was extracted from herbarium, silica gel-dried, or air-dried leaf samples.

Appendix 1 lists the voucher information, specimen numbers, type of material used, and GenBank numbers. Fresh (1–2.0 g) or dried (0.1–0.2 g) leaf material was ground into a fine powder and incubated according to the shortened 2× CTAB procedure of Doyle and Doyle (1987). Proteins were removed with SEVAG (24:1, chloroform:isoamyl alcohol), followed by an isopropanol precipitation. Purified DNA was stored at –80°C.

RBCL GENE AMPLIFICATION

The amplification of the *rbcL* gene was performed either as one complete piece using the forward primer that matched the first 20 base pairs (1F-ATGTCAC-CACAAACAGAAAC) of the exon and a reverse primer (1460R-TCCTTTTACTAAAAGATTGGGCC-GAG) that matched a downstream control site (Olmstead et al., 1992) or as two overlapping pieces using three additional internal primers, 636F (GCGTTGGAGAGATCGTTTCT), 724R (TCCGATG-TACCYGCAGTTGC), and 1368R (CTTTCCAAATTT-CACAAGCA GCA). When primer mismatch occurred, primer combinations changed accordingly. The polymerase chain reaction (PCR) was performed using standard protocols. The Thermal Cycler 480 (Perkin Elmer Inc., Waltham, Massachusetts, U.S.A.) was programmed to perform 25 cycles of denaturation at 94°C for 1 min., primer annealing at 50°C for 30 sec., and extension at 72°C for 1 min. Slight modifications to optimize the reaction conditions were found to be necessary for some taxa; that is, the MgCl₂ and bovine serum albumin (BSA) concentrations were varied. Products were purified using QIAGEN QIAquick PCR purification kit (QIAGEN Inc., Chatsworth, California, U.S.A.) following protocols provided by the manufacturer.

ITS GENE AMPLIFICATION

The amplification of the ITS gene was performed using oligonucleotide primers 17SE (ACGAATT-CATGGTCCGGTGAAGTGTTCCG) and 26SE (TAGAATTCCCGGTTCCGCTCGCCGTTAC) as described by Sun et al. (1994). The PCR was performed using standard protocols; however, the DNA template, depending on the concentration, was diluted 1:10 or 1:100. The thermal cycler was programmed to perform an initial one cycle of denaturation at 95°C for 2 min. followed by 24 cycles of 95°C for 30 sec., 55°C for 30 sec., and 72°C for 1 min. 30 sec. This was followed by 10 min. extension at 72°C. The same procedures described in the preceding section were followed after completion of the PCR.

CYCLE SEQUENCING

Cleaned products were then directly sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase, FS (PerkinElmer Inc.) following the manufacturer's protocol. Unincorporated dye terminators were removed using 3 mol/L sodium acetate and ethanol precipitation as recommended by the manufacturer. Samples were then loaded into a gel on an ABI 373A DNA Automated Sequencer. Raw sequencing data were edited and assembled using the program Lasergene 2 (DNASTAR, Inc., Madison, Wisconsin, U.S.A.). The alignments were constructed using the CLUSTAL option. All assemblies and initial alignments underwent subsequent manual editing.

MOLECULAR ALIGNMENT

The conserved nature of the *rbcl* gene with no insertions and deletions (indels) made this a fairly simple task. For ITS alignments, the indel coding method of Simmons and Ochoterena (2000) was adapted, while ignoring autapomorphies.

MOLECULAR DATA ANALYSIS

Phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford, 2000). Uninformative characters were excluded from all analyses. Heuristic searches were conducted initially under the unordered and equal weighting criteria of Fitch parsimony (Fitch, 1971) with 100 random sequence additions, tree bisection-reconnection (TBR) branch swapping and MUPARS in effect, steepest descent on. Ten trees were held in each step. Strict consensus trees were obtained, and branch lengths and tree scores were calculated using ACCTRAN (accelerated transformation optimization). The initial trees found with equal (Fitch) weights were used as the basis for successive weighting. Successive weighting was carried out using the rescaled consistency index. Reweighting was continued until the same tree length was obtained in two successive rounds. Relative support for individual clades was estimated with the bootstrap method (Felsenstein, 1985). One thousand pseudoreplicates were performed with uninformative characters excluded. To reduce bootstrap search times, branches were collapsed if their minimum length was zero ("amb-"), and no more than 2000 trees were saved per search.

Bayesian analyses were performed using MrBayes 3.1.1 (Ronquist et al., 2005) on the individual molecular and combined molecular, and morphological and molecular data sets. The substitution model for each DNA region was selected with MrModeltest

2.1 (Ronquist et al., 2005) under the Akaike Information Criterion (AIC). The parameters for the Bayesian analyses were as follows: nst = 6; rates = gamma; set autoclose = yes; mcmc npngen = 1000000; prinfewq = 100; samplefreq = 10; savebriens = yes. In the combined Bayesian analysis (molecular and morphology), a mixed-model approach was used. The combined data were partitioned, and the above model of evolution was used for the molecular data sets. In each case, the first 25% of the trees were regarded as "burn in" and omitted, and the majority rule consensus tree was obtained in PAUP* from the remaining trees.

SELECTION OF MORPHOLOGICAL CHARACTERS

The coding method "C" in Kitching et al. (1998) has been adopted in this study. In this independent coding method, every attribute is given a separate character, and inapplicable observations for the absence of the feature are accommodated by using question marks. In the case of filaments, the presence and absence have been coded as a separate character, whereas the length, presence of hairs, and form in bud were coded as three independent characters. Inapplicable observations for the absence of the feature were denoted with question marks to overcome the problem of overscoring the state's absence when many different characters are perceived as connected to a feature that is absent from some taxa (Maddison, 1993).

Potentially useful characters had to be discarded because data for only a few genera were available. Many of the characters are readily coded as binary; however, patterns of morphological variations found in the taxa necessitate the use of unordered multistate characters.

When genera are scored as terminal taxa, a large amount of polymorphism can be introduced into the data set because of the highly variable nature of some taxa. In this study, to avoid problems associated with the inclusion of polymorphism in the data set (Nixon & Davies, 1991), subgeneric-level taxa have been included for the genera *Licania* and *Magnistipula*.

INGROUP SAMPLING

All the 17 genera in the Chrysobalanaceae have been coded for their morphological characters. Characters were scored to the extent possible from herbarium specimens taken primarily from the collection at Royal Botanic Gardens, Kew. This information was supplemented with published observations from the literature (Prance, 1963, 1972, 1989; Prance & White, 1988) and personal knowledge of one of the authors (Prance). Characters were selected by reviewing previous work and searching for variations that had not been previously analyzed.

PREPARATION OF MORPHOLOGICAL MATERIAL

Flowers. Dried herbarium material was revived by boiling in water and then dissected and observed using a dissecting microscope.

Leaves/leaf architecture. Leaf venation (primary vein [midvein], secondary, tertiary, and quaternary veins) was coded by clearing leaves and observing their patterns. One hundred ninety-seven herbarium leaf samples were used in this study. The materials were obtained from the herbarium at the Royal Botanic Gardens, Kew. Vouchers used in this study are listed in Appendix 2. The protocol for leaf clearing was modified from Radford et al. (1974) for herbarium materials. Terminology in general follows Hickey (1979).

MORPHOLOGICAL DATA ANALYSIS

The data matrix consisted of a total of 50 characters. The matrix is presented in Table 2, and the list of characters and character states used in the morphological analysis is presented in Appendix 3. Of the 50 characters, 38 are binary (in which 16 of them are coded as simple absence/presence) and 12 are multistate. All analyses were conducted using PAUP* as stated above.

RESULTS

For the parsimony analyses, the potentially informative phylogenetic characters in each data set, the number of trees, the tree length, the consistency indices, the retention indices, and the number of branches with bootstrap values along with the number of branches with bootstrap values greater than 70% are found in Table 3. For Bayesian analysis, the number of branches with posterior probability values greater than 90% and 95% are found in Table 3.

LARGER *rbcl*

The length of the analyzed *rbcl* gene is 1382 bp in all samples, and no gaps were required for alignment. Approximately 45 bp at the beginning and 17 bp at the end were deleted from the *rbcl* matrix. There were 984 invariant characters. The final data matrix consisted of 398 variable characters, of which 241 were parsimony informative. Unweighted pairwise sequence divergence among species of Chrysobalanaceae ranges from 0.2% to 2.9%; that between species of Chrysobalanaceae and the outgroups ranges from 4.5% to 10.5%. Sequence divergence among the outgroups ranges from 1.6% to 19.0%. Mean percentage G + C content is 45.0%.

A heuristic search under the Fitch criterion yielded 338 equally most parsimonious trees (MPTs) of 749 steps with a consistency index (CI) of 0.49 and a retention index (RI) of 0.74. Successive weighting produced 139 MPTs of 223.22 steps with a CI of 0.67 and an RI of 0.89, which corresponds to a Fitch length of 753 steps, a CI of 0.49, and an RI of 0.74. The unambiguous transition:transversion ratio was 318:200 (1.59).

Only the Bayesian tree is shown in Figure 1 due to space limitations. All trees recovered well-supported and monophyletic Chrysobalanaceae (100% Fitch equal and successively weighted trees and the Bayesian tree). In addition, all trees resolved *Euphronia* as sister to Chrysobalanaceae with *Dichapetalum* and *Tapura* (Dichapetalaceae) and *Trigonía* (Trigonaceae) as sister to *Euphronia*. *Balanops* (Balanopaceae) is placed as the sister taxon to the above grouping. Although the family groupings are poorly resolved on the strict consensus Fitch tree, the successive weighting and the Bayesian tree slightly increased both the resolution and clade support. In the strict consensus Fitch tree, the generic relationships are poorly resolved, consisting of only three clades: *Couepia*–*Couepia robusta* Huber (bootstrap support [BS] 88%); *Maranthes*–(*Grangeria*–*Magnistipula*); and *Hunga*–*Neocarya*. The successively weighted tree is mostly a polytomy with only three major clades present: *Hirtella*–*Hirtella bicornis* Mart. & Zucc.–*Licania*–(*Couepia*–*Couepia robusta* [BS 96%]); *Maranthes*–(*Grangeria*–*Magnistipula*) (BS 72%); and *Chrysobalanus*–(*Hunga*–*Neocarya* [BS 93%]).

In the Bayesian analysis, the generic relationships are poorly resolved, consisting of a polytomy with four clades: *Couepia*–*Couepia robusta* (100%); *Dactyladenia*–*Trichocarya* (71%); *Maranthes*–(*Grangeria*–*Magnistipula* [74%]) (98%); and *Hunga*–*Neocarya* (58%).

SMALLER *rbcl*

In this analysis, there were 1143 invariant characters. The final data matrix consisted of 239 variable characters, of which 118 were parsimony informative. Uninformative characters were excluded from the analysis. Unweighted pairwise sequence divergence among species of Chrysobalanaceae ranges from 0.2% to 2.9%; that between species of Chrysobalanaceae and the outgroups ranges from 4.5% to 7.3%. Sequence divergence among the outgroups ranges from 2.3% to 7.0%. Mean percentage G + C content is 45.0%.

A heuristic search under the Fitch criterion yielded 577 equally MPTs of 182 steps with a CI of 0.65 and an RI of 0.73. Successive weighting produced 139 MPTs of 88.81 steps with a CI of 0.89 and an RI of

Table 2. Matrix of morphological characters. See Appendix 3 for character list; ? = missing data or inapplicable character states.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Chrysobalanus</i>	1	0	0	0,1	1	1	0	1	1	0	0	1	0	1
<i>Grangeria</i>	1	0	0	0	0	1	0	1	1	0	1	1	1	1
<i>Licania</i> subg. <i>Moquilea</i>	0	0	0	0,1	0	1	0	1	1	0	0	1	0	1
<i>Licania</i> subg. <i>Parinariopsis</i>	0	0	0,1	1	0	1	1	1	1	1	0	1	0	1
<i>Licania</i> subg. <i>Licania</i>	0	0	0,1	1	0	1	0,1	1	1	0	0	1	0	1
<i>Licania</i> subg. <i>Afrolicania</i>	1	0	0	0	0	1	0	1	1	0	1	1	0	1
<i>Licania</i> subg. <i>Angelesia</i>	1	0	0	1	0	1	0	1	1	0	0	1	0	2
<i>Licania</i> subg. <i>Leptobalanus</i>	0,1	0	0,1	0,1	0	1	0	1	1	0	0	1	0	1
<i>Parastemon</i>	1	0	0	0	0	0	0	1	1	0	0	1	0	1
<i>Bafodeya</i>	0	0	1	1	0	1	0	1	1	0	0	1	0	2
<i>Exellodendron</i>	1	0	0	0,1	0	1	0	1	1	0	0	1	0	1
<i>Hunga</i>	1	0	0	0,1	0	1	0	0,1	1	0	0	1	0	1
<i>Neocarya</i>	1	0	1	1	0	1	0	1	1	1	0	1	0	2
<i>Parinari</i>	1	0	1	1	0	1	0	1	1	1	0	1	0	1
<i>Acioa</i>	1	0	0	0	0	1	0	1	1	0	0	1	2	2
<i>Couepia</i>	1	0	0,1	0,1	0	1	0,1	1	1	0	0	1	0,2	1
<i>Maranthes</i>	1	0	0	0,1	0	1	0	1	1	0	0	1	0,2	1
<i>Atuna</i>	1	0	0	0	0	1	0	1	1	0	0	1	0	1
<i>Dactyladenia</i>	1	0	0	0,1	0	1	0	1	1	0	0	1	0,2	2
<i>Hirtella</i>	1	0	0	0,1	1	1	0	1	1	0	0,1	1	0	1
<i>Kostermanthus</i>	1	1	0	0	0	1	0	1	1	0	0	1	2	2
<i>Magnistipula</i> subg. <i>Magnistipula</i>	1	0	0	0	0	1	0	1	1	0	0	1	0	2
<i>Magnistipula</i> subg. <i>Pellegriniella</i>	1	0	0	0	0	1	0	1	1	0	0	1	0	2
<i>Magnistipula</i> subg. <i>Tolmiella</i>	1	0	0	0	0	1	0	1	1	0	0	1	1	2
<i>Dichapetalum</i>	1	0	0	0,1	0	0	0	1	1	0	0	0	0	0,1
<i>Tapura</i>	1	0	0	0	0	0	0	1	1	0	0	0	0,2	0
<i>Stephanopodium</i>	1	0	0	0	0	0	0	1	1	0	0	0	0	2

0.93, which corresponds to a Fitch length of 182 steps, a CI of 0.65, and an RI of 0.73. The unambiguous transition:transversion ratio was 157 to 196:78 to 105 (2.01 to 0.018).

Figure 2A shows the successively weighted strict consensus tree, and Figure 2B shows the Bayesian tree. All trees recovered revealed a monophyletic Chrysobalanaceae. The family is well supported (100% bootstrap Fitch equal and successively weighted trees) as a monophyletic group on both trees. Although the family groupings are poorly resolved on the strict consensus Fitch tree, the successive weighting increased both the resolution and clade support. Only the clade *Licania*–*Chrysobalanus* is supported on the equal-weighted tree; on the weighted tree, however, four major clades are supported. The number and distribution of unambiguous transition:transversion ratios calculated are similar on both equal-weighted (157 to 196:78 to 105) and successively weighted (157 to 174:78 to 101) trees.

On the strict consensus of the successively weighted *rbcl* tree, four major clades can be recognized. Clade A with *Atuna* is sister to a poly-

tomy consisting of clades B, C, D, *Acioa*, *Bafodeya*, *Kostermanthus*, *Neocarya*, and *Parinari*. Clade B is an unresolved polytomy containing *Grangeria*–*Magnistipula*–*Maranthes* (BS 69%). Clade C contains an unresolved polytomy of *Dactyladenia*–*Exellodendron*–*Hunga*. Clade D is a monophyletic clade bearing *Hirtella*–*Couepia*–(*Licania*–*Chrysobalanus* [BS 85%]) supported by 84% bootstrap.

In the Bayesian analysis, the family is monophyletic and well supported (100%). The generic relationships are poorly resolved with only three clades present: *Chrysobalanus*–*Licania* (51%); *Couepia*–*Hirtella* (60%); and *Maranthes*–(*Grangeria*–*Magnistipula* [64%]) (96%). There are no hard conflicts between the Bayesian tree and parsimony trees.

ITS

Boundaries of the coding and spacer regions were estimated by comparison with sequences of the ITS region in Baldwin (1992). Sequences were easily aligned, with only *Dichapetalum* being slightly difficult in containing a large indel, and cloning was not

Table 2. Continued.

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Chrysobalanus</i>	1	2	1	1	0	0	1	0	1	1	0	2	1	1	0
<i>Grangeria</i>	1	0	0	?	0	1	1	0	0,1	1	0,1	2	1	0	0
<i>Licania</i> subg. <i>Moquilea</i>	0,1	1	1	1	?	0	1	0	1	1	0	2	0,1	0	0
<i>Licania</i> subg. <i>Parinariopsis</i>	1	1	1	0,1	2	1	1	1	1	1	0	2	0	1	0
<i>Licania</i> subg. <i>Licania</i>	0,1	1	1	1	2	0,1	1	0	0	1	0,1	0	0,1	0,1	0
<i>Licania</i> subg. <i>Afrolicania</i>	0	?	1	1	1	0	0	0	1	1	0	0	0	0	0
<i>Licania</i> subg. <i>Angelesia</i>	1	1	1	0,1	1	0	1	1	0	1	0,1	0	0	0	0
<i>Licania</i> subg. <i>Leptobalanus</i>	0,1	1	1	1	1	0	1	0	0,1	1	0	2	0	0	0
<i>Parastemon</i>	1	1	1	0,1	0	0	1	0	0	1	0,1	0	0	0	0
<i>Bafodeya</i>	1	1	1	0,1	1	2	1	1	0	1	1	1	1	0	1
<i>Exellodendron</i>	1	1	1	0,1	2	1	1	1	0	1	1	1	1	0	0
<i>Hunga</i>	1	0	1	0,1	2	1	1	1	0	1	1	0	0	0	0
<i>Neocarya</i>	1	0	1	0	0	2	1	1	1	1	1	2	1	0	1
<i>Parinari</i>	1	0,1	1	0,1	2	1	1	1	0	1	1	0	1	0	1
<i>Acioa</i>	1	2	1	0	2	2	1	1	1	1	1	2	2	0	2
<i>Couepia</i>	1	1	1	0,1	2	1	1	1	1	1	0,1	2	1	0	1
<i>Maranthes</i>	1	1	0	?	2	1	1	0	1	1	0	2	1	0	2
<i>Atuna</i>	1	1	1	0,1	2	1	1	1	1	1	1	2	2	0	2
<i>Dactyladenia</i>	1	1	1	0	2	2	1	1	1	1	1	2	2	0	2
<i>Hirtella</i>	1	0	1	0	0	1	1	1	0	1	1	2	0	0	2
<i>Kostermantus</i>	1	2	1	1	0	2	1	0	1	1	1	2	2	0	0
<i>Magnistipula</i> subg. <i>Magnistipula</i>	1	2	1	0,1	2	2	1	1	0	1	1	1	1	0	0
<i>Magnistipula</i> subg. <i>Pellegriniella</i>	1	2	1	0	2	2	1	1	0	1	1	0	1	0	0
<i>Magnistipula</i> subg. <i>Tolmiella</i>	1	2	1	0,1	2	1	1	0	0	1	1	2	1	1	0
<i>Dichapetalum</i>	1	0	?	?	?	0	1	?	0	1	0	1,2	0	0	1
<i>Tapura</i>	1	2	?	?	?	0	1	?	0	1	1	2	3	1	1
<i>Stephanopodium</i>	1	2	?	?	?	0	1	?	0	0	0	3	3	?	?

necessary. The length of the ITS region ranges from 763–779 bp among species of Chrysobalanaceae and from 754–784 bp among the outgroups. The ingroup length of ITS-1 ranges from 319–332 bp, that of the 5.8S subunit from 160–162 bp, and that of ITS-2 from 278–290 bp. The outgroup length of ITS-1 ranges from 292–342 bp, that of the 5.8S subunit from 163–165 bp, and that of ITS-2 from 277–304 bp. Multiple sequence alignment of Chrysobalanaceae and all outgroups resulted in a data matrix of 833 characters, of which 168 (20.2%) include at least one accession with a gap (92 of 332 positions [27.7%] in ITS-1, 5 of 162 positions [3.1%] in the 5.8S subunit, and 71 of 290 positions [24.5%] in ITS-2). Unweighted pairwise sequence divergence among species of Chrysobalanaceae ranges from 5.8% to 16.5% in ITS-1, from 5.7% to 19.9% in ITS-2, and from 0.0% to 5.6% in the 5.8S; that between species of Chrysobalanaceae and the outgroups ranges from 27.8% to 34.9% in ITS-1, from 36.3% to 79.1% in ITS-2, and from 0.3% to 7.3% in the 5.8S subunit. Mean percentage G + C content is 59.2%.

Multiple sequence alignment of Chrysobalanaceae and the two selected realigned outgroups resulted in a

matrix of 833 characters. Of the 833 positions constituting the aligned ITS sequences, 411 were variable and 234 were parsimony informative. Uninformative characters were excluded from the analysis. Of the 234 parsimony informative characters, 58 were missing ambiguous. The inclusion of gap coding resulted in more homoplasy and a lack of resolution; therefore, gap coding was not used in the following results.

A heuristic search under the Fitch criterion yielded 18 MPTs of 751 steps with a CI of 0.57 and an RI of 0.40. Successive weighting produced two equally parsimonious trees of 288.3 steps with a CI of 0.65 and an RI of 0.59, which corresponds to a Fitch length of 749 steps, a CI of 0.57, and an RI of 0.39. The transition:transversion ratio was 371:281 (1.320). Figure 3A shows the successively weighted strict consensus, and Figure 3B shows the Bayesian tree.

The ITS sequence data, both equal-weighted and weighted analysis, indicate a monophyletic Chrysobalanaceae (79% bootstrap for the Fitch tree and 100% for the successively weighted tree). Successive weighting trees are more resolved and have more support with fewer trees with a greater number of unambiguous

Table 2. Continued.

	30	31	32	33	34	35	36	37	38	39	40	41	42
<i>Chrysobalanus</i>	0	0	0	1	1	1	1	1	0	0	0	0	1
<i>Grangeria</i>	0,1	2	0	1	1	0	1	0	0	0	1	?	?
<i>Licania</i> subg. <i>Moquilea</i>	0	0	0	1	1	1	1	1	0	?	?	?	?
<i>Licania</i> subg. <i>Parinariopsis</i>	0	1	0	1	1	1	1	1	0	?	?	?	?
<i>Licania</i> subg. <i>Licania</i>	0,1	0	0	1	1	1	1	0,1	0,1	?	?	?	?
<i>Licania</i> subg. <i>Afrolicania</i>	0	0	0	1	1	0	1	1	0	?	?	?	?
<i>Licania</i> subg. <i>Angelesia</i>	0	0	0	0	1	1	1	1	0	?	?	?	?
<i>Licania</i> subg. <i>Leptobalanus</i>	0	0	0	1	1	1	1	1	0	1	1	?	?
<i>Parastemon</i>	0,1	0	0	1	0	1	1	0	0	0	0	0	0
<i>Bafodeya</i>	1	1	1	1	1	1	1	0	1	0	1	?	?
<i>Exellodendron</i>	1	2	1	1	1	1	1	1	0	0	1	?	?
<i>Hunga</i>	1	1	1	1	1	1	1	1	0	0	1	?	?
<i>Neocarya</i>	1	2	1	1	1	1	1	1	1	1	?	0	0
<i>Parinari</i>	1	1	1	1	1	1	1	1	1	1	?	0	1
<i>Acioa</i>	1	2	0	1	1	1	1	0	0	0	1	1	?
<i>Couepia</i>	0,1	2	0	1	1	1	1	0,1	0	1	1	0	1
<i>Maranthes</i>	1	2	1	1	1	1	1	1	0	1	1	1	0
<i>Atuna</i>	1	2	1	1	1	1	1	0	1	1	1	0	?
<i>Dactyladenia</i>	1	2	0	1	1	1	1	0	1	1	1	0	?
<i>Hirtella</i>	1	2	0	1	1	1	1	1	0	0	1	0	1
<i>Kostermanthus</i>	1	2	0	1	1	1	1	0	1	?	0	?	?
<i>Magnistipula</i> subg. <i>Magnistipula</i>	1	2	0	1	1	1	1	1	1	?	1	0	0
<i>Magnistipula</i> subg. <i>Pellegriniella</i>	1	2	1	1	1	1	1	1	1	?	?	0	?
<i>Magnistipula</i> subg. <i>Tolmiella</i>	1	2	0	1	1	1	1	1	1	?	?	0	?
<i>Dichapetalum</i>	0	0	2,3	1	0,1	1	0	0	1	?	?	?	?
<i>Tapura</i>	1	0	3	1	0,1	1	0	0	1	?	?	?	?
<i>Stephanopodium</i>	0	0	2,3	1	1	1	0	0	1	?	?	?	?

synapomorphies than the equally weighted trees. Therefore, the results are discussed based on the weighted strict consensus tree. A total of 50 unambiguous synapomorphies supports the family using the weighted strict consensus trees.

The family is monophyletic (BS 100%) with low internal resolution in the successively weighted strict consensus tree. The internal family clade contains *Atuna*, *Kostermanthus*, and a polytomy of clade A, *Dactyladenia*, *Acioa*, and clade B. Clade A has *Neocarya*–*Parinari* (BS 96%) sister to (*Bafodeya*–*Grangeria*)–(*Magnistipula*–*Maranthes*). Clade B has *Exellodendron*–*Hunga* (BS 52%), which is sister to (*Chrysobalanus*–*Couepia*)–(*Hirtella*–*Licania* [BS 67%]).

In the Bayesian analysis, the family is monophyletic and well supported (100%). The internal family clade contains a polytomy of clade A sister to *Dactyladenia*–(*Kostermanthus*–*Atuna* [87%]) (93%), *Acioa*–*Couepia*–*Chrysobalanus*–(*Exellodendron*–*Hunga* [100%]), and *Hirtella*–*Licania* (57%). Clade A contains *Neocarya*–*Parinari* (100%) sister to *Bafodeya*–*Grangeria*–(*Magnistipula*–*Maranthes* [100%]) (75%). There is no conflict between the supported clades of the Bayesian and the parsimony topologies.

COMBINED DATA

Following the modified methods outlined by Mason-Gamer and Kellogg (1996), we considered the data sets combinable. In all analyses, the family is monophyletic with 100% bootstrap support. Only one node is in conflict between the *rbcL* and the ITS topologies with bootstrap values greater than 75%. In the *rbcL* parsimonious tree (Fig. 2A), *Chrysobalanus*–*Licania* are held together with 85% bootstrap support forming a polytomy with *Couepia*–*Hirtella* (BS 84%), whereas in the ITS tree (Fig. 3A) these two taxa are grouped as *Chrysobalanus*–*Couepia* sister to *Hirtella*–*Licania* (BS 67%) with less than 70% bootstrap support. There are no hard conflicts between these trees; therefore, the incongruence is interpreted as being due to chance and the data sets were combined.

Within the Bayesian analysis, one position is in conflict between the *rbcL* and the ITS topologies with posterior probabilities greater than 95%. In the *rbcL* tree, *Maranthes*–(*Grangeria*–*Magnistipula* [64%]) (96%) (Fig. 2B) form a group, whereas in the ITS tree, *Bafodeya*–*Grangeria*–(*Magnistipula*–*Maranthes* [100%]) (75%) form a clade (Fig. 3B). The only conflict is due to the lack of resolution and having

Table 2. Continued.

	43	44	45	46	47	48	49	50
<i>Chrysobalanus</i>	1	0	0	1	1	0	1	0
<i>Grangeria</i>	1	0	1	1	0	0	1	0
<i>Licania</i> subg. <i>Moquilea</i>	1	0	0	0	0	0	1	0
<i>Licania</i> subg. <i>Parinariopsis</i>	1	0	0	1	0	0	1	0
<i>Licania</i> subg. <i>Licania</i>	1	0	0	1	0,1	0	1	0
<i>Licania</i> subg. <i>Afrolicania</i>	1	1	0	1	1	0	1	0
<i>Licania</i> subg. <i>Angelesia</i>	1	0	0	1	0	0	1	0
<i>Licania</i> subg. <i>Leptobalanus</i>	?	0	0	0	0	0	1	0
<i>Parastemon</i>	0	1	0	0	0	0	1	0
<i>Bafodeya</i>	?	1	0	1	0	0	1	0
<i>Exellodendron</i>	?	0	0	1	0	0	1	0
<i>Hunga</i>	?	1	0	1	0	0	1	0
<i>Neocarya</i>	1	1	0	0	2	1	1	0
<i>Parinari</i>	1	1	1	1	0	2	0	0
<i>Acioa</i>	0	1	1	1	2	0	1	0
<i>Couepia</i>	1	1	1	1	1	2	0	0
<i>Maranthes</i>	0	1	0	0	0	0	1	0
<i>Atuna</i>	1	1	0	1	0	0	1	0
<i>Dactyladenia</i>	0	1	0	1	0	0	1	0,1
<i>Hirtella</i>	1	0	0	1	1	0	1	0
<i>Kostermantus</i>	?	1	1	0	0,1	0	1	0
<i>Magnistipula</i> subg. <i>Magnistipula</i>	0	1	1	1	0	0	1	0
<i>Magnistipula</i> subg. <i>Pellegriniella</i>	0	1	0	1	0	0	1	0
<i>Magnistipula</i> subg. <i>Tolmiella</i>	0	1	1	0	1	0	1	0
<i>Dichapetalum</i>	?	1	0	1	0	0	1	0
<i>Tapura</i>	?	1	0	1	0	0	1	0
<i>Stephanopodium</i>	?	1	0	1	0	0	1	0

Bafodeya missing in the *rbcL* topology. Therefore, there are no real hard conflicts between these trees. In the combined molecular data set, only two outgroups, *Dichapetalum* and *Euphronia*, are in common. Difficulty in either amplification or the limitation of plant material caused this reduction in the number of outgroups. The total combined data set consisted of 2215 characters, of which 280 characters were parsimony informative.

A heuristic search under the Fitch criterion yielded nine MPTs of 850 steps. The consensus tree had a CI of 0.56 and an RI of 0.41 (trees not shown). Successive weighting of this tree produced one equally parsimonious tree of 332.87 steps with a CI of 0.66 and an RI of 0.62, which corresponds to a Fitch length of 850, a CI of 0.56, and an RI of 0.41. The successively weighted strict consensus tree is more resolved than the equal-weighted tree.

Table 3. Comparison of results from the parsimony analysis of the larger *rbcL*, *rbcL*, ITS, and a combination of all data.

							Bayesian values		
Total no. of characters/No. of informative characters		No. of trees	Length	CI	RI	BS ¹	BS ≥ 70% ¹	PP ≥ 90% ²	PP ≥ 95% ²
Large <i>rbcL</i>	1382/241	139	753	0.49	0.74	19	16	16	16
<i>rbcL</i>	1382/118	139	182	0.65	0.73	9	8	4	4
ITS	833/234	2	749	0.57	0.39	4	2	5	4
Combined	2265/315	2	987	0.53	0.38	5	3	7	5

BS, bootstrap support; CI, consistency index; RI, retention index.
¹ For parsimony analysis, the BS (the number of branches that contained bootstrap support ≥ 50%) and BS ≥ 70 (the number of branches that contained bootstrap support ≥ 70%) were included.
² For the Bayesian analysis, the posterior probabilities ≥ 90% (the number of branches that contained posterior probability values ≥ 90%) and posterior probabilities ≥ 95% (the number of branches that contained posterior probability values ≥ 95%) were included.

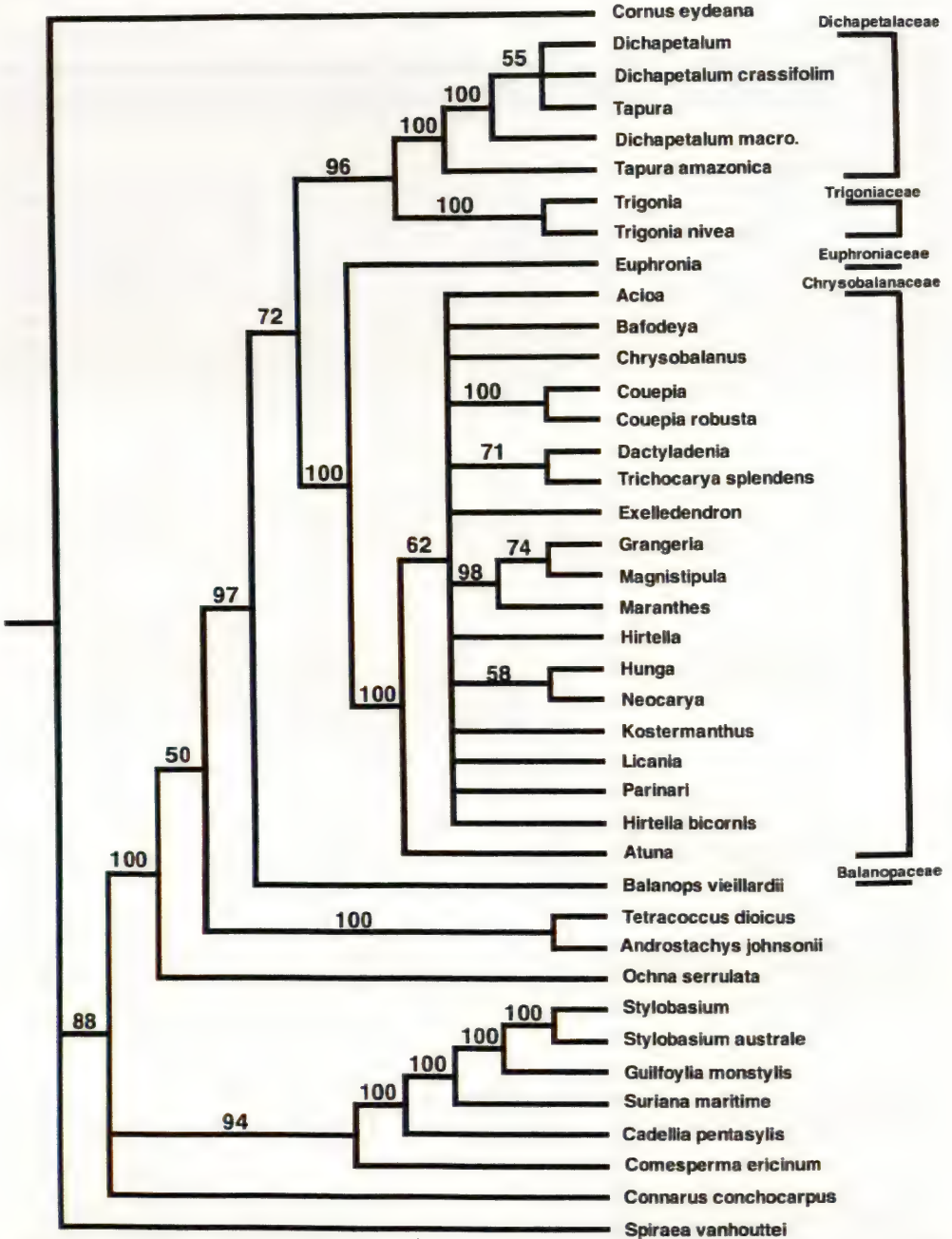


Figure 1. Bayesian consensus tree from the larger *rbcL* analysis. Numbers above nodes are posterior probability values.

therefore the former is the tree used in the following discussion.

The family is monophyletic with strong bootstrap support (100%). The internal family clade contains *Atuna*, *Kostermanthus*, clade A, *Dactyladenia*, and

clade B. Clade A has *Neocarya*–*Parinari* (BS 67%) sister to (*Bafodeya*–*Grangeria*)–(*Magnistipula*–*Maranthes* [BS 73%]). Clade B has *Acioa* basal to *Exelodendron*–*Hunga* (BS 99%), which is sister to (*Chrysobalanus*–*Couepia*)–(*Hirtella*–*Licania*).

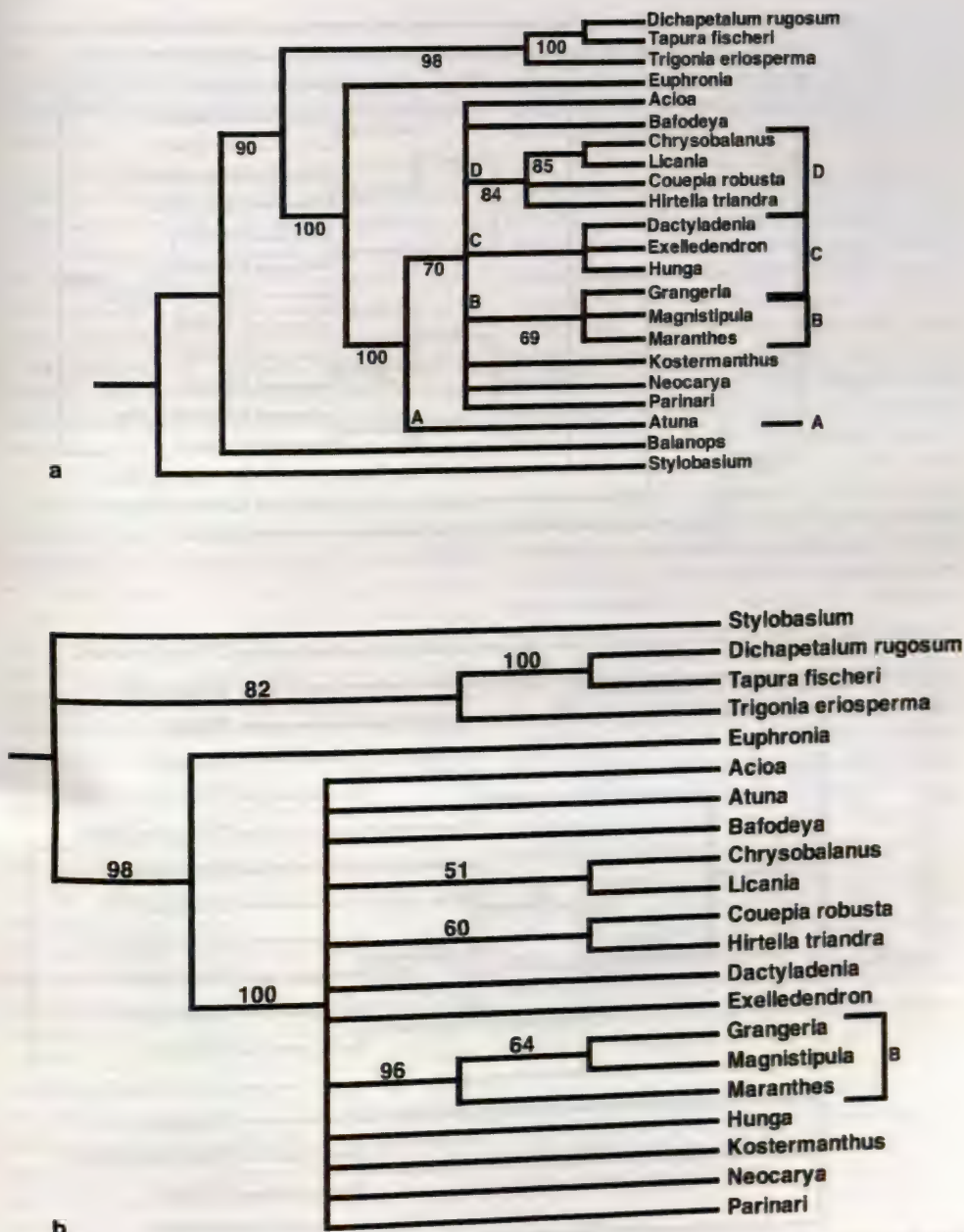


Figure 2. —A. The successively weighted strict consensus tree of 139 most parsimonious trees obtained from the *rbcl* data from the Chrysobalanaceae (length = 118 steps, CI = 0.65, RI = 0.73). Numbers below nodes are bootstrap values. —B. Bayesian consensus tree with posterior probability values.

In the Bayesian analysis, the family is monophyletic with good posterior probability values (100%). The internal family clade contains a polytomy of four clades, part of clade A from above, clade B, *Neocarya*–*Parinari* (100%), and *Dactyladenia*–(*Kostermanthus*–*Atuna* [92%]) (90%). Clade A contains

Magnistipula–*Maranthes* (BS 100%), *Grangeria* (94%), and *Bafodeya* (70%). Clade B has *Acioa* sister to *Exelledendron*–*Hunga* (BS 100%), which is sister to *Chrysobalanus*–*Couepia*–(*Hirtella*–*Licania* [56%]) (85%). There are no conflicts between the supported clades of the Bayesian and the parsimony topologies.

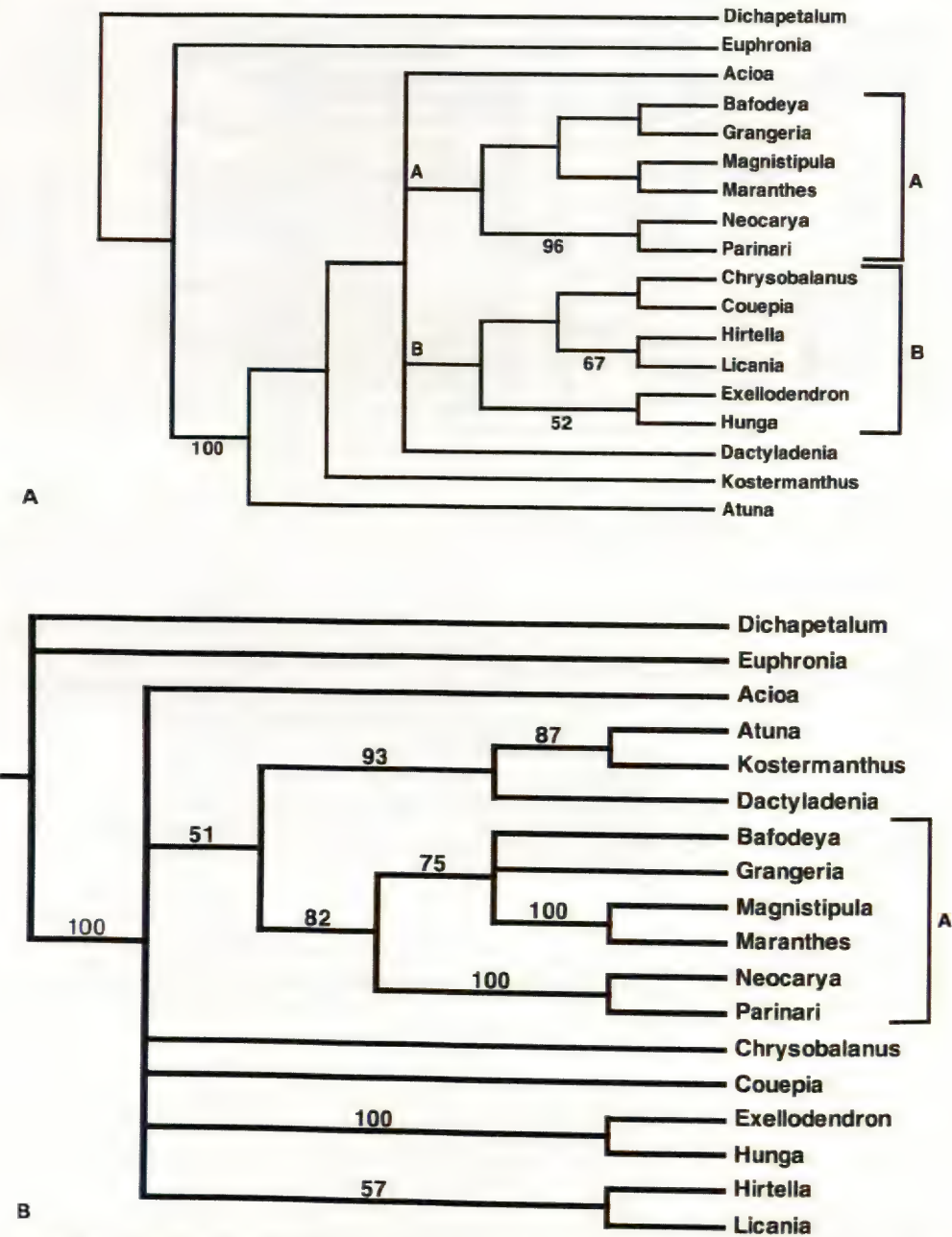


Figure 3. —A. The successively weighted strict consensus tree of the two most parsimonious trees (length = 749 steps, CI = 0.57, RI = 0.39) obtained from the ITS data from the Chrysobalanaceae. Numbers below nodes are bootstrap values. —B. Bayesian consensus tree with posterior probability values.

According to a modified Mason-Gamer and Kellogg (1996) method, the combined molecular and morphological data sets were considered combinable. An independent morphological and molecular analysis

was completed under parsimony. Although the morphological topology is considerably different from the combined molecular topology, there is little bootstrap support on the morphological tree. There-

fore, conflicts between the data sets with bootstrap values greater than 75% do not exist.

The total combined data set consisted of 2265 characters, of which 315 characters were parsimony informative.

A heuristic search under the Fitch criterion yielded 12 MPTs of 985 steps. The consensus tree had a CI of 0.533 and an RI of 0.386. Successive weighting based on this tree produced two equally parsimonious trees of 255.66 steps with a CI of 0.741 and an RI of 0.702, which corresponds to a Fitch length of 987, a CI of 0.532, and an RI of 0.383. One of these trees is shown as a phylogram in Figure 4A. The successively weighted trees are more resolved than the equal-weighted trees and contain higher bootstrap support than the equal-weighted trees. Therefore the following discussion uses the successively weighted tree.

Only the outgroup taxon *Dichapetalum* is common to all three data sets. *Euphronia* was also included, even though the morphological data have been coded as missing data due to the lack of adequate material.

Chrysobalanaceae is monophyletic with strong bootstrap support (100%). *Atuna* is sister to the remaining taxa. The next clade consists of *Kostermanthus*, sister to *Acioa* and *Dactyladenia*. This clade is sister to two larger clades. The first contains *Exellodendron-Hunga* (BS 100%) sister to (*Chrysobalanus-Couepia*)-(*Hirtella-Licania*) (BS 60%). The second clade has *Neocarya-Parinari* (BS 70%) sister to (*Bafodeya-Grangeria*)-(*Magnistipula-Maranthes*). The primary difference between the combined tree and the combined molecular tree is the clade containing *Kostermanthus*.

In the Bayesian analysis (Fig. 4B), the family is monophyletic and well supported (100%). The internal family clade contains a polytomy of three clades, clade A, clade B, and *Dactyladenia*-(*Kostermanthus-Atuna* [BS 82%]) (BS 65%). Clade A has a weakly supported polytomy of *Bafodeya* with *Grangeria*-(*Magnistipula-Maranthes* [BS 98%]) (BS 98%), and *Neocarya-Parinari* (BS 100%). Clade B has *Acioa* sister to *Exellodendron-Hunga* (BS 100%), which is sister to a polytomy of *Chrysobalanus-Couepia-Hirtella-Licania* (BS 90%). There are no hard conflicts between the supported clades of the Bayesian and the parsimony topologies; in fact, they are very similar except for the positions of *Atuna* and *Acioa*.

DISCUSSION

RELATIONSHIPS OF CHRYSOBALANACEAE

A recent study using 18S rDNA, *rbcl*, and *atpB* sequence data (Soltis et al., 2000) showed that even

though Chrysobalanaceae had been closely associated with Rosaceae by previous workers, it is not closely related to the family. Both independent (*rbcl*) and combined analyses support the sister-group relationship of Chrysobalanaceae to Trigoniaceae (Chase et al., 1993), to Dichapetalaceae (Soltis et al., 2000), to Balanopaceae (Soltis et al., 2005), and to *Euphronia* (Litt & Chase, 1999), as one of several subclades within Malpighiales. A relationship between the Chrysobalanaceae, Trigoniaceae, and Dichapetalaceae based on nonmolecular data had been proposed by Hallier (1908, 1921), although few phylogenetically useful morphological features were produced from this study. Recently, Litt and Chase (1999) attempted to find morphological evidence to support the relationships between Chrysobalanaceae, Trigoniaceae, Dichapetalaceae, and Euphroniaceae. Litt and Chase (1999) found no obvious morphological synapomorphies, although there was strong *rbcl* support for these relationships. Our molecular results from both the independent and combined analysis support the sister-group relationships of Chrysobalanaceae and *Euphronia*. The larger *rbcl* analysis supports *Euphronia* as the sister taxon to Chrysobalanaceae with *Dichapetalum* and *Tapura* (Dichapetalaceae) and *Trigonia* (Trigoniaceae) sister to *Euphronia*. *Balanops* (Balanopaceae) occurs as the sister taxon to the above grouping. Stevens (2003) reported that these plants have vestured pits, paracytic stomata, usually zygomorphic flowers, and tenuinucellate ovules. In addition, Chrysobalanaceae, Dichapetalaceae, and Trigoniaceae have two ovules per carpel.

The work of Matthews and Endress (2008) uncovered new floral features for Chrysobalanaceae, Dichapetalaceae, Euphroniaceae, and Trigoniaceae. Matthews and Endress (2008) found a number of floral synapomorphies for each of the families and concluded that the phylogenetic topology for these families still remained unresolved mainly due to a lack of molecular data. Our study found Chrysobalanaceae as a well-supported family, sister to Euphroniaceae, while Dichapetalaceae and Trigoniaceae formed a well-supported sister grouping.

MONOPHYLY OF CHRYSOBALANACEAE

The position of the Chrysobalanaceae varied greatly among past analyses. The genera of the Chrysobalanaceae traditionally have often been linked with the Rosaceae (Jussieu, 1789; de Candolle, 1825; Meisner, 1837–1838; Hooker, 1865; Focke, 1891) or occasionally with the Oleaceae (de Candolle, 1842). Prance (1963) clearly placed the group as a family distinct from the Rosaceae based on wood anatomy, pollen morphological studies, and blastogeny taken in

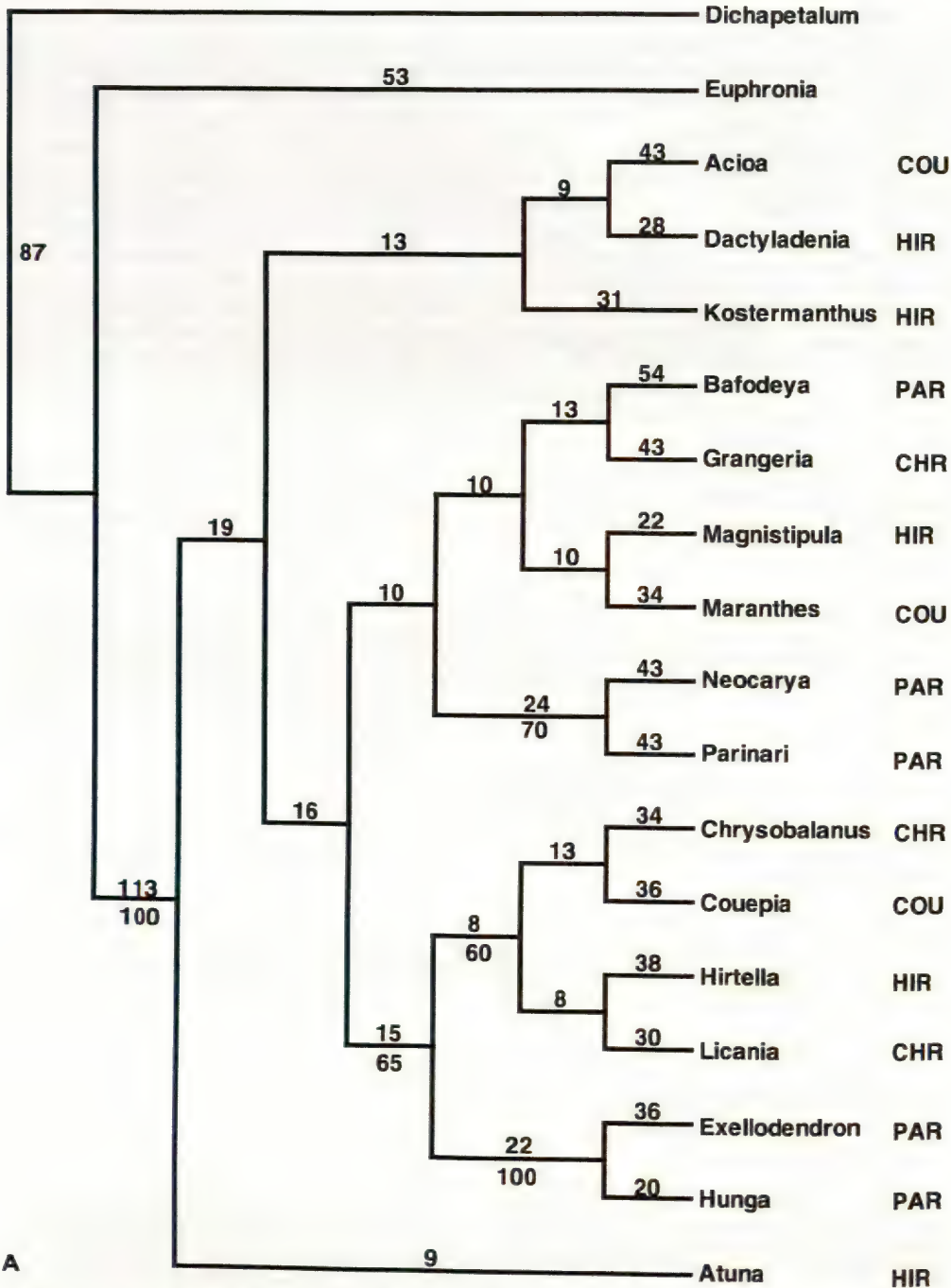
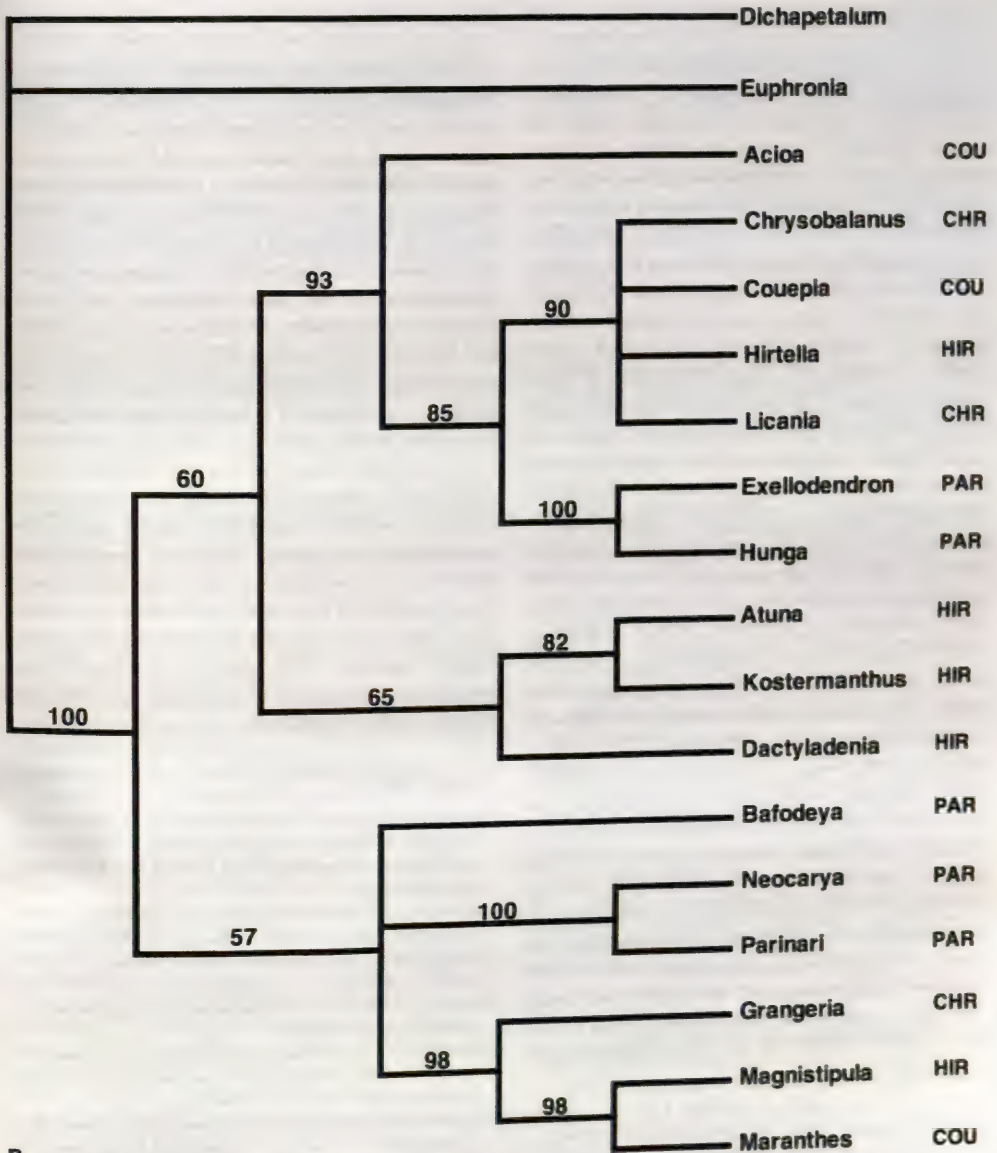


Figure 4. —A. One of the most parsimonious trees arbitrarily selected from the two equally optimal trees from the combined analysis (length = 987 steps, CI = 0.53, RI = 0.38). Numbers above each branch are branch lengths. Numbers below each branch are bootstrap values. The four tribes as outlined by Prance and White (1988) are labeled as follows: Chrysobalaneae = CHR, Couepieae = COU, Parinarieae = PAR, and Hirtelleae = HIR. —B. Bayesian consensus tree from the combined analysis. The four tribes as outlined by Prance and White (1988) are labeled as follows: Chrysobalaneae = CHR, Couepieae = COU, Parinarieae = PAR, and Hirtelleae = HIR. Numbers above nodes are posterior probability values.



B

Figure 4. Continued.

conjunction with leaf anatomy and chemistry. Our results show that the Chrysobalanaceae clade is strongly supported by the independent and combined morphological and molecular analyses. The family has three unambiguous morphological synapomorphies, i.e., presence of silica bodies, a receptacle tube (= hypanthium), and a gynobasic style. Individually, these characters occur in various groups of eudicots; however, the combination of these features is distinctive. Other families, the Lamiaceae and Boraginaceae, are currently recognized as having a

gynobasic style, but these families do not have silica bodies widely present. Although the given features occur sporadically in other families, they are diagnostically significant in this family because the combination of these features is taxonomically restricted. The combination of these features suggests a close phylogenetic relationship between these genera. It is clear from the phylogenetic studies that the gynobasic condition evolved within the family clade. These features also clearly separate Chrysobalanaceae from the Rosaceae.

THE RELATIONSHIP OF THE TRIBES WITHIN CHRYSOBALANACEAE

None of the four traditionally defined tribes are monophyletic in the combined or independent analysis. The best-resolved trees came from the Bayesian analysis of the combined data set, which included 16 taxa of Chrysobalanaceae and two outgroups. The morphological character states were traced onto the combined morphological and molecular tree using MacClade 4.0 (Maddison & Maddison, 2000) in order to find non-homoplasious characters. This combined analysis contained internal support for most of the major clades, which we have named "clade 1," "clade 2," "clade 3," "clade 4," and "clade 5." Clade 1 consists of *Exellodendron* and *Hunga* (100%) from the tribe Parinarieae and has no morphological synapomorphies. This clade is sister to clade 2, which consists of a polytomy of *Chrysobalanus* and *Licania* of the tribe Chrysobalanaceae, *Couepia* of the Couepieae, and *Hirtella* of the tribe Hirtelleae. This clade has support (90%) and has one unambiguous synapomorphy of the presence of leaf venation of secondary vein type 1. Clade 1 and clade 2 (85%) have no known morphological synapomorphies. *Acioa* is sister to the above-mentioned taxa (93%). This clade is sister to clade 3. Clade 3 contains *Kostermanthus* and *Atuna* (82%) and is sister to *Dactyladenia*, all of the tribe Hirtelleae. Clade 3 has one unambiguous synapomorphy: leaf papillae present. The above clades are sister to a polytomy of *Bafodeya*, clade 4, and clade 5 (57%). Clade 4 contains *Neocarya* and *Parinari* (100%), belonging to the Parinarieae with two morphological synapomorphies: the presence of stomatal crypts and large bracts. Clade 5 includes *Grangeria* of the tribe Couepieae, which is sister to *Magnistipula*, and *Maranthes* of the tribes Chrysobalanaceae and Hirtelleae. Clade 5 (98%) has no known morphological synapomorphies.

TYPES OF ANALYSIS

Two primary types of analysis were completed: parsimony analysis using successive weighting and Bayesian analysis. The combined molecular and morphological data sets had similar topologies with two of the three major clades in agreement. There were three clades with bootstrap support values greater than 70% in the successively weighted tree and five clades with the posterior probability values equal to or greater than 95% in the Bayesian tree. The successively weighted tree provided similar topology with less support than the Bayesian analysis, indicating that within this data set the successively weighted tree is more conservative.

TAXONOMIC CONCLUSIONS

Chrysobalanaceae, as traditionally circumscribed, is monophyletic. *Euphronia* of the Euphroniaceae is the closest relative to the Chrysobalanaceae. Under the current system, these families would be placed in the eurosids (Rosidae, in Cantino et al., 2007) in the order Malpighiales (APG II, 2003; Davis & Chase, 2004; Davis et al., 2005; Tokuoka & Tobe, 2006; Soltis et al., 2007). None of the four traditionally recognized tribes, Chrysobalanaceae, Couepieae, Parinarieae, and Hirtelleae, are monophyletic. Morphological data indicate that the genera *Licania* and *Magnistipula* are not monophyletic, and further work using molecular data and more taxa is needed to confirm these findings and restructure generic limits. This study did find that there are two to three major clades within this family. The relationships among these clades are supported by moderate posterior probability values and a few morphological characters. Additional morphological features need to be examined to find more unambiguous synapomorphies. Future studies will include additional DNA regions and examination of additional morphological characters. Only when more molecular and morphological characters are examined can taxonomic realignments be formalized.

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APPENDIX 1. Voucher information, specimen numbers, type of materials extracted (G = GenBank, S = silica, H = herbarium), and GenBank accession numbers used in the molecular study. Herbarium specimens are deposited at the Royal Botanic Gardens, Kew (K), the Missouri Botanical Garden (MO), and in the private collections of Morton (*).

Taxon	Specimen	Voucher	<i>rbcL</i>	ITS
Family Balanopaceae				
<i>Balanops vieillardii</i> Baill.	G	Litt & Chase, 1999	AF089760	
Family Chrysobalanaceae				
<i>Acioa guianensis</i> Aubl.	S	Rio de Janeiro, Brazil, <i>Prance 30841</i> (K)	GQ424473	GQ424453
<i>Atuna racemosa</i> Raf. subsp. <i>racemosa</i>	S	Bogor, Indonesia, <i>Morton 89</i> (*)	GQ424474	GQ424454
<i>Bafodeya benna</i> (Scott-Elliott) Prance ex F. White	S	Guinea, West Africa, <i>Col M. Saidu s.n.</i> , 1997 (K)	GQ424475	GQ424455
<i>Chrysobalanus icaco</i> L.	S	Bogor, Indonesia, <i>Morton 64</i> (*)	GQ424476	
<i>C. icaco</i>	S	Dominican Republic, <i>Prance 30833</i> (K)		GQ424456
<i>Couepia obovata</i> Ducke	S	French Guiana, <i>D. Sabatier & M. F. Prevost 3125</i> (MO)	GQ424477	
<i>C. parillo</i> DC.	S	Reserva Florestal Ducke, Brazil, <i>R. C. Forzza 305</i> (MO)		GQ424457
<i>C. robusta</i> Huber	G	Litt & Chase, 1999	AF089757	
<i>Dactyladenia pallescens</i> (Baill.) Prance & F. White	H	Gabon, <i>McPherson 16317</i> (K)	GQ424478	GQ424458
<i>Exellodendron barbatum</i> (Ducke) Prance	S	Brazil, <i>Hopkins & Vicentini 1209</i> (K)	GQ424479	GQ424459
<i>Grangeria borbonica</i> Lam.	H	Madagascar, <i>Derleth 61</i> (K)	GQ424480	GQ424460
<i>Hirtella bicornis</i> Mart. & Zucc.	G	Litt & Chase, 1999	AF089756	
<i>H. triandra</i> Sw.	S	Dominican Republic, <i>S. R. Hill 29095</i> (K)	GQ424481	GQ424461
<i>Hunga gerontogea</i> (Schltr.) Prance	H	New Caledonia, <i>McPherson 6093</i> (MO)	GQ424482	GQ424462
<i>Kostermantus robustus</i> Prance	S	Sarawak, Borneo, <i>Morton tree1099</i> (K)	GQ424483	GQ424463
<i>Licania tomentosa</i> (Benth.) Fritsch	S	Bogor, Indonesia, <i>Morton 97</i> (*)	GQ424484	GQ424464
<i>Magnistipula butayi</i> De Wild.	H	Zaire, Africa, <i>T. B. Hart 1362</i> (K)		GQ424465
<i>M. conrautana</i> Engl.	H	Cameroon, Africa, <i>A. J. M. Leeuwenberg 9572</i> (K)	GQ424485	
<i>Maranthes glabra</i> (Oliv.) Prance	S	Cameroon, Africa, <i>J. J. Bos 7352</i> (K)	GQ424486	GQ424466
<i>Neocarya macrophylla</i> (Sabine) Prance ex F. White	S	Senegal, Dakar, <i>Goudiaby & Sambou</i> , 1998 (K)	GQ424487	GQ424467
<i>Parinari sumatrana</i> (Jack) Benth.	S	Bogor, Indonesia, <i>Morton 134</i> (*)	GQ424488	GQ424468
Family Connaraceae				
<i>Connarus conchocarpus</i> F. Muell.	G	Ferando et al., 1993	L29493	

APPENDIX 1. Continued.

Taxon	Specimen	Voucher	rbL	ITS
Family Cornaceae				
<i>Cornus eydeana</i> Q. Y. Xiang & Y. M. Shui	G	Xiang et al., 2005	AY243874	
Family Dichapetalaceae				
<i>Dichapetalum crassifolium</i> Chodat	G	Savolainen et al., 1994	X69733	
<i>D. macrocarpum</i> Engl.	G	Litt & Chase, 1999	AF089764	
<i>D. rugosum</i> (Vahl) Prance	S	Brazil, Spruce 623 (K)	GQ424469	GQ424451
<i>Tapura amazonica</i> Poepp. & Endl.	G	Litt & Chase, 1999	AF089763	
<i>T. fischeri</i> Engl.	H	Lowveld Botanical Garden, South Africa, Prance 30831 (K)	GQ424471	
Family Euphorbiaceae				
<i>Androstachys johnsonii</i> Prain	G	Savolainen et al., 2000	AJ402922	
<i>Tetracoccus dioicus</i> Parry	G	Davis et al., 2005	AY788190	
Family Euphroniaceae				
<i>Euphronia guianensis</i> (R. H. Schomb.) Hallier f.	S	Bolívar, Venezuela, Berry et al. 6562 (MO)	GQ424470	GQ424452
Family Ochnaceae				
<i>Ochna serrulata</i> (Hochst.) Walp.	G	Fay et al., 1997	Z75273	
Family Polygalaceae				
<i>Comesperma ericinum</i> DC.	G	Fernando et al., 1993	L29492	
Family Rosaceae				
<i>Spiraea vanhouttei</i> (Briot) Carrière	G	Morgan & Soltis, 1993	L11206	
Family Stylobasiaceae				
<i>Stylobasium australe</i> (Hook.) Prance	G	Fernando et al., 1993	U07679	
<i>S. spatulatum</i> Desf.	G	Soltis et al., 1993	U06828	
Family Surianaceae				
<i>Cadellia pentastylis</i> F. Muell.	G	Fernando et al., 1993	L29491	
<i>Guilfoylia monostylis</i> F. Muell.	G	Fernando et al., 1993	L29494	
<i>Suriana maritima</i> L.	G	Fernando et al., 1993	U07680	
Family Trigoniaceae				
<i>Trigonia eriosperma</i> (Lam.) Fromm & E. Santos	S	Rio de Janeiro, Brazil, Prance 30842 (K)	GQ424472	
<i>T. nivea</i> Cambess.	G	Litt & Chase, 1999	AF089761	

APPENDIX 2. Taxon and voucher information used in the leaf morphology study. All material used are from herbarium specimens deposited at the Royal Botanic Gardens, Kew (K).

Taxon	Voucher
Family Chrysobalanaceae	
<i>Acioa schultesii</i> Maguire	Venezuela, Pedro 6661
<i>Atuna penangiana</i> Kosterm.	[Indonesia] Malay, Cockburn FRI 8202
<i>A. racemosa</i> Raf.	[Indonesia] Malay, Johor FRI 37727
<i>Bafodeya benna</i> (Scott-Elliot) Prance ex F. White	Guinea, Col. M. Saidu s.n. 1997
<i>Chrysobalanus icaco</i> L.	Ivory Coast [Côte d'Ivoire], Oldeman 692
<i>C. venezuelanus</i> Prance	Venezuela, Cardozo 2536
<i>Couepia guianensis</i> Aubl.	Brazil, Pacheco 38
<i>C. magnoliifolia</i> Benth. ex Hook. f.	Brazil, Campbell P20859
<i>C. parillo</i> DC.	Brazil, Hoffman 3584

APPENDIX 2. Continued.

Taxon	Voucher
<i>C. platycalyx</i> Cuatrec.	Costa Rica, <i>Gerardo Herrera</i> 5375
<i>C. uiti</i> (Mart. & Zucc.) Benth. ex Hook. f.	Brazil, <i>Guedes</i> 3081
<i>Dactyladenia gillettii</i> (De Wild.) Prance & F. White	Gabon, <i>McPherson</i> 03-28-1992
<i>D. letestui</i> (Letouzey) Prance & F. White	Cameroon, <i>Breteler</i> 1646
<i>D. scabrifolia</i> (Hua) Prance & F. White	Guiana, <i>Brown</i> 2627
<i>D. whytei</i> (Stapf) Prance & F. White	Sierra Leone, <i>Deighton</i> 5104
<i>Exellodendron barbatum</i> (Ducke) Prance	Guyana, <i>Jacobs</i> 1928
<i>E. coriaceum</i> (Benth.) Prance	Guyana, <i>Maas</i> 7601
<i>Grangeria borbonica</i> Lam.	Mauritius, <i>Bosser</i> 21872
<i>G. porosa</i> Boivin ex Baill.	Madagascar, <i>Phillipson</i> 1909
<i>Hirtella bullata</i> Benth.	Guyana, <i>Henkel</i> 251
<i>H. gracilipes</i> (Hook. f.) Prance	Brazil, <i>Harley</i> 21649
<i>H. pilosissima</i> Mart. & Zucc.	Brazil, <i>Thomas</i> 4969
<i>H. tocantina</i> Ducke	Brazil, <i>Carvalho</i> 6103
<i>H. zanzibarica</i> Oliv.	Mozambique, <i>Finley</i> 2178
<i>Hunga mackeeana</i> Prance	New Caledonia, North Province, <i>Mackee</i> 19
<i>H. minutiflora</i> (Baker f.) Prance	New Caledonia, North Province, <i>Mackee</i> 17561
<i>H. myrsinoides</i> (Schltr.) Prance	New Caledonia, North Province, <i>Mackee</i> 23803
<i>H. rhamnoides</i> (Guillaumin) Prance	New Caledonia, North Province, <i>Mackee</i> 29322
<i>Kostermanthus heteropetalus</i> (Scort. ex King) Prance	[Indonesia] Malay, <i>Cockburn FRI</i> 7966
<i>K. heteropetalus</i>	Brunei, <i>Coode</i> 7708
<i>Licania</i> Aubl. subg. <i>Licania</i>	
<i>L. arborea</i> Seem.	Nicaragua, <i>Moreno</i> 22875
<i>L. boyanii</i> Tutin	Guiana, <i>Clarke</i> 532
<i>L. buxifolia</i> Sandwith	Guiana, <i>Jacobs</i> 1981
<i>L. coriacea</i> Benth.	Guiana, <i>Mutuhnick</i> 747
<i>L. cuprea</i> Sandwith	Guiana, <i>Pipoly</i> 9640
<i>L. cyathodes</i> Benoist	French Guiana, <i>Wachendeims</i> 141
<i>L. dealbata</i> Hook. f.	Brazil, <i>Almeida</i> 275
<i>L. discolor</i> Pilg.	Brazil, <i>Dick</i> 139
<i>L. heteromorpha</i> Benth.	Venezuela, <i>Aymard</i> 6393
<i>L. heteromorpha</i> Benth.	Brazil, <i>Sothers</i> 690
<i>L. hypoleuca</i> Benth.	Costa Rica, <i>Herrera</i> 4829
<i>L. impressa</i> Prance	Brazil, <i>Sothers</i> 894
<i>L. irwinii</i> Prance	Guiana, <i>Cremers</i> 10639
<i>L. kunthiana</i> Hook. f.	Brazil, <i>Millikein</i> , 11 Feb. 1988
<i>L. lasserii</i> Maguire	French Guyana, <i>Hoffman</i> 1183
<i>L. latifolia</i> Benth. ex Hook. f.	Guiana, <i>Gillespie</i> 2933
<i>L. laxiflora</i> Fritsch	Brazil, <i>Kukle</i> 78
<i>L. majuscula</i> Sagot	Guiana, <i>Maas</i> , 3 Oct. 1988
<i>L. mollis</i> Benth.	Brazil, <i>Prance</i> 29931
<i>L. oblongifolia</i> Standl.	Brazil, <i>Thomas</i> 6507
<i>L. polita</i> Spruce ex Hook. f.	Brazil, <i>Ferreira</i> 7460
<i>L. reticulata</i> Prance	Brazil, <i>Ribeiro</i> 1168
<i>L. urceolaris</i> Hook. f.	Brazil, <i>Prance</i> 14158
<i>Licania</i> subg. <i>Leptobalanus</i> Benth.	
<i>L. sclerophylla</i> (Mart. ex Hook. f.) Fritsch	Brazil, <i>Pereira</i> 2839
<i>L. sprucei</i> (Hook. f.) Fritsch	Guyana, <i>Henkal</i> 5135
<i>Licania</i> subg. <i>Moquilea</i> (Aubl.) Prance	
<i>L. arborea</i> Seem.	Nicaragua, <i>Moreno</i> 22875
<i>L. granvillei</i> Prance	French Guiana, <i>Mori</i> 20783
<i>L. michauxii</i> Prance	Florida, U.S.A., <i>Foster</i> 13556
<i>L. unguiculata</i> Prance	[Brazil] Ducke Reserve, <i>Vicentini A.</i> 746

APPENDIX 2. Continued.

Taxon	Voucher
<i>Licania</i> subg. <i>Parinariopsis</i> Huber	
<i>L. licaniiiflora</i> (Sagot) S. F. Blake	Brazil, Oldeman, 10 June 1966
<i>Licania</i> subg. <i>Angelesia</i> (Korth.) Prance & F. White	
<i>L. splendens</i> (Korth.) Prance	[Indonesia] Malaysia, Ridley 261
<i>Licania</i> subg. <i>Afrolicania</i> (Mildbr.) F. White & Prance	
<i>L. elaeosperma</i> (Mildbr.) Prance & F. White	Cameroon, De Wilde & de Wilde-Duyffes 1850
<i>Magnistipula</i> Engl. subg. <i>Magnistipula</i>	
<i>M. butayei</i> De Wild.	Tanzania, Boaler 643
<i>M. sapinii</i> De Wild.	Congo Republic, Lisowski et al. 13220
<i>Magnistipula</i> subg. <i>Pellegriniella</i> (Hauman) Prance	
<i>M. tessmannii</i> (Engl.) Prance	Gabon, Wilks 1572
<i>Magnistipula</i> subg. <i>Tolmiella</i> F. White	
<i>Magnistipula tamenaka</i> (Capuron) F. White	Madagascar, Schatz 3329
<i>Maranthes corymbosa</i> Blume	Philippines, Ridsdale & Baquiran ISV 489
<i>M. floribunda</i> (Baker) F. White	Zaire, Lisowski 54259
<i>M. glabra</i> (Oliv.) Prance	Ivory Coast [Côte d'Ivoire], Oldeman 857
<i>M. kerstingii</i> (Engl.) Prance ex F. White	Nigeria, Chapman 4082
<i>Neocarya macrophylla</i> (Sabine) Prance ex F. White	Sierra Leone, Deighton 5302
<i>Parastemon urophyllus</i> (Wall. ex A. DC.) A. DC.	Singapore, Symington 35516
<i>P. versteeghii</i> Merr. & L. M. Perry	Guinea, Streimann NGF 45157
<i>Parinari gigantea</i> Kosterm.	[Indonesia] Bogor, Kosterman 34697
<i>P. insularum</i> A. Gray	[Fiji] Rotuma Island, John 19168
<i>P. montana</i> Aubl.	Brazil, Rabelo 3555
<i>P. oblongifolia</i> Hook. f.	Malaysia, Kochummen 78747
<i>P. sprucei</i> Hook. f.	Brazil, Kawasaki 220
Family Dichapetalaceae	
<i>Dichapetalum albidum</i> A. Chev. ex Pellegr.	Sierra Leone, Thomas 3173
<i>D. angolense</i> Chodat	Belgian Congo, Derred 442
<i>D. barteri</i> Engl.	Nigeria, Edwin Ujor 15292
<i>D. brenesii</i> Standl.	Costa Rica, Bello 192
<i>D. crassifolium</i> Chodat	Sierra Leone, Deighton 3633
<i>D. geloniodes</i> (Roxb.) Engl.	South India, Ridsdale 764
<i>D. pallidum</i> Engl.	Ivory Coast [Côte d'Ivoire], Breteler 5364
<i>D. setosum</i> Leenh.	Brunei, Bygrave et al. 4
<i>D. timoriense</i> (DC.) Boerl.	[Indonesia] Kalimantan, Sidiyasa 803
<i>Stephanopodium blanchetianum</i> Baill. in Mart.	Brazil, Pinheiro 1713
<i>S. costaricense</i> Prance	Costa Rica, Robles 2066
<i>S. estrellense</i> Baill. in Mart.	Brazil, Martinelli et al. 10006
<i>S. sessile</i> Rizzini	Brazil, Comm et al. 18910
<i>Tapura acreana</i> (Ule) Rizzini	Peru, Vasquez 3754
<i>T. africana</i> Engl.	Cameroon, Thomas 2470
<i>T. capitulifera</i> Baill.	British Guiana, JB 522, 4 Mar. 1953
<i>T. coriacea</i> J. F. Macbr.	Brazil, Ferreira et al. 10853
<i>T. fischeri</i> Engl.	Zaire, Van der Ben 1326
<i>T. guianensis</i> Aubl.	Suriname, Lindeman et al. 566

APPENDIX 3. List of characters and character states used in the morphological analysis of Chrysobalanaceae. The data matrix with 50 binary and multistate characters for the 26 taxa included in this study is given in Table 2. Thirty of the characters were scored from the generic description of Prance and White (1988) and are in agreement with the Chappill (1992) matrix. Eleven of the characters (*) were included from the tribal descriptions of Prance and White (1988).

1. Lamina glands: Lamina glands occur in almost all species and were scored as absent = 0 or present = 1. The majority of species of *Parinari* possess two circular glands and several small marginal or submarginal glands, so this character was scored as present for *Parinari*.

2. Leaf papillae: Leaf papillae on veins were coded as absent = 0 or present = 1. The majority of species of *Licania* and *Couepia* do not possess leaf papillae; this feature was scored as absent for these genera.

3. Stomatal crypts: The veins on the abaxial surface of some specimens are extremely prominent and form a dense network occupying more than half of the surface so that the stomata are confined to relatively small, sunken crypts. These cavities are filled at the mouth with woolly hairs. This feature was scored as absent = 0 or present = 1. Most species of *Parinari* contain stomatal crypts; this feature was coded as present for the genus.

4. Leaf trichomes: Trichomes on leaves were scored as absent = 0 or present = 1. Foliar hairs are unicellular and simple (Prance, 1972). In *Couepia*, *Exellodendron*, and *Maranthes*, they are long, thin walled, flexuose, and arachnoid and form a weblike covering on the leaves. By contrast, in *Hirtella*, *Magnistipula*, and most species of *Dactyladenia*, they are either straight and stiff or strigose and setose (Prance & White, 1988). Even though the family contains variation in trichome types, details for all the genera are not available. This character was coded as absent or present for this study. Only a few species of *Magnistipula* possess trichomes, and therefore this feature was coded as absent for the genus. In *Licania* subg. *Afrolicania* (Mildbr.) F. White & Prance, the trichomes are sometimes present in young leaves, which become glabrous very soon; therefore, this feature was coded as absent.

5. Epidermal cells: Non-mucilaginous = 0; mucilaginous = 1.

6. Silica bodies in the epidermis: Silicified membranes are universal, with silica bodies present in the epidermal cells and surrounding the leaf veins (Prance, 1972). This feature was scored as absent = 0 or present = 1.

7. Petiolar glands: Absent = 0; present = 1.

8. Stipules: Absent = 0; present = 1. Stipules are nearly always present but occasionally are small and caducous. In some species of *Parinari*, the stipules reach a length of 7 cm. In *Atuna*, they are prominently keeled and represent a unique feature in the family. Intrapetiolar stipules occur in *Maranthes* and some species of *Licania* and *Magnistipula*. In other species of *Magnistipula* and in *L. latistipula* Prance, the stipules are lateral and foliaceous. In *Magnistipula zenkeri* Engl., they are sometimes up to 5 cm long and inflated. Although there seems to be a variation in the type and size of the stipules, they have been tentatively coded as absent and present in this study due to the difficulty in observing them in the herbarium specimens. Because the majority of species of *Hunga* possess stipules, the character is coded as present for the genus. According to de Souza (1979) and Prance (1972), stipules are absent in *Chrysobalanus*; however, according to Prance and White (1988), they

are present and caducous. The character has been scored as present for *Chrysobalanus*.

9. Bracts: The distinction between bracts and bracteoles cannot always be drawn on mature specimens. Therefore, bracts and bracteoles together have been scored as bracts. Absent = 0; present = 1.

10. Bract size: Bracts and bracteoles are usually small, but in a few species they are relatively large and enclose small groups of developing flowers. This character was scored as small = 0 or large = 1.

11. Bract glands: Absent = 0; present = 1. Most taxa have eglandular bracts and bracteoles, but in several species of *Dactyladenia*, *Hirtella*, *Grangeria*, and *Licania* subg. *Afrolicania*, they are glandular and in some cases visible even to the naked eye. Because the majority of species of *Acioa*, *Magnistipula*, and *Couepia* lack glands, the character was scored as absent for these genera.

12. Receptacle tube: Absent = 0; present = 1.

13. Sepals: Acute = 0; obtuse = 1; round = 2.

***14. Sepal shape:** Equal = 0; subequal = 1; unequal = 2.

15. Petals: Absent = 0; present = 1.

16. Petal length: Shorter than the calyx = 0; equaling the calyx = 1; longer than the calyx = 2.

17. Receptacle hairs: Absent = 0; present = 1.

18. Receptacle hairs: Hairs on the inner surface of the receptacle tube can be present at mouth = 0 or throughout the interior = 1.

***19. Receptacle tube length:** Shorter than calyx = 0; equal to calyx = 1; longer than calyx = 2. The length of the receptacle tube was determined in relation to the calyx and, other than few exceptions (*Maranthes corymbosa*, *Couepia platycalyx*—receptacle shorter than calyx), most of the genera could be coded into discrete states.

***20. Floral symmetry:** Actinomorphic = 0; slightly zygomorphic = 1; strongly zygomorphic = 2. The floral symmetry can be described as actinomorphic (apart from the lateral style) to strongly zygomorphic.

21. Sexual habit: Unisexual = 0; bisexual = 1.

***22. Retrose hairs in throat:** A distinct feature of the receptacle tube is the presence of retrose hairs at the throat, which was scored as absent = 0 or present = 1.

***23. Stamen number:** The number of stamens varies from two in *Parastemon urophyllus* to more than 300 in some species of *Couepia*. This character was divided into two discrete states and scored as 10 or less = 0 or more than 11 = 1. In *Licania*, which has only a very few species with nine to 12 or 10 to 11 stamens, this character was coded as 10 or less. In *Acioa*, *Atuna*, and *Dactyladenia*, the few species with a stamen number between 10 and 20, 10 and 25, and 10 and 75 have been scored as more than 11.

24. Filaments: Absent = 0; present = 1.

***25. Fertile stamen placement:** When all the stamens are fertile, they form a complete or an almost complete circle around the entrance to the flower. In the case with the presence of staminodes, the fertile stamens are inserted unilaterally opposite the carpel. This feature was scored as a complete or almost complete circle = 0 or unilaterally opposite the carpel = 1.

***26. Filament length:** The length of the filaments can vary from much shorter than the calyx = 0, as long as the calyx = 1, or longer than the calyx = 2.

***27. Filament union:** In a majority of genera, the filaments are free or united at the base for less than 1/3 of their length. In the case of the three genera that have filaments united more than 1/3 of their length, the union forms a conspicuous staminal ligule. In the case of *Dactyladenia*, the ligule is far exerted and much longer

than the combined length of the calyx and receptacle tube. In *Acioa edulis* Prance, a dubious species in the genus, the filaments are free. However, because the majority is ligulately connate, this character has been scored at the generic level as united more than 1/3 the length for this data set. Filaments free = 0; united at base = 1; united more than 1/3 its length = 2.

28. Filament hairs: Absent = 0; present = 1.

***29. Filaments in bud:** In those genera with far-exserted stamens, the filaments are coiled in the bud. In a few others, they are slightly undulate. This feature was scored as not coiled = 0, undulate = 1, or coiled = 2.

***30. Staminodes:** Staminodes may be absent = 0 or present = 1. Because the genus *Acioa* has rudimentary staminodes, it is scored as present for the genus.

***31. Ovary insertion:** The position of the ovary can be at the base of the receptacle, halfway up the receptacle tube, or at the mouth of the receptacle tube. The three states were scored as base of receptacle = 0, lateral = 1, or mouth of the receptacle tube = 2. In most species of *Hirtella*, the ovary is inserted at the mouth of the receptacle tube; therefore, it has been scored as mouth of the receptacle tube for the genus.

32. Ovary locules: The ovary is unilocular, but in some members the ovary can be bilocular or trilocular. This feature is scored as unilocular = 0, bilocular-false dissepiment = 1, true bilocular = 2, or trilocular = 3.

33. Ovary hairs: Absent = 0; present = 1.

34. Stigma: The stigma may be deeply lobed = 0 or scarcely lobed = 1.

35. Style hairs: Absent = 0; present = 1.

36. Gynobasic style: Absent = 0; present = 1.

37. Drupe: The fruit is a drupe, which can be fleshy = 0 or dry = 1.

38. Epicarp: Smooth = 0; rough = 1.

39. Endocarp surface: Smooth = 0; rough = 1.

40. Endocarp interior: Glabrous = 0; hairy = 1.

41. Germination: Germination has been studied in 12 genera (Prance, 1972, and references therein) and provides

useful taxonomic characters, especially for generic delimitation. The character was coded as cryptocotylar = 0 or phanerocotylar = 1 following Duke (1965, 1969).

42. Cataphylls: The scale leaves preceding the foliage leaves can be absent = 0 or present = 1.

43. First pair of eophylls: The first few leaves with green, expanded laminae are opposite = 0 or alternate = 1.

44. Rays: Uniseriate = 0; biseriate = 1.

45. Primary vein size: Primary vein size was determined midway between the leaf apex and base as to the ratio of vein width (vw) to leaf width (lw) and defined as $vw:lw \times 100\%$. $> 2 \text{ mm} = 0$; $\geq 2.1 \text{ mm} = 1$.

46. Secondary vein angle: Angle divergence of the secondary veins was measured above the point of branching. Angle $45^\circ = 0$; angle $\geq 46^\circ = 1$.

47. Secondary veins—variation in angle divergence: Variation in the angle of divergence can be uniform or nonuniform with upper more acute than lower or upper more obtuse than lower. This was scored as uniform = 0, nonuniform type 1 = 1, and nonuniform type 2 = 2.

48. Secondary vein course: The radiation pattern of the secondary veins was grouped into three patterns that can be easily distinguished from one another. For type 1, the secondary veins gradually turn up and divide at half their length, and division of the secondary veins that join the adjacent secondary veins is visible. For type 2, only some secondary veins divide and do not show quite such a distinct pattern, such as the *Neocarya* type. For type 3, the secondary veins turn up very close to the margin, and the division of the secondary veins that join the adjacent secondary veins is obscure. Type 1 = 0; type 2 = 1; type 3 = 2.

49. Tertiary vein pattern: Parallel = 0; reticulate = 1.

50. Areoles: Areoles are the smallest area of the leaf tissue surrounded by veins, which, taken together, form a contiguous field over most of the area of the leaf. Incomplete = 0; well developed = 1.